The Bone Tissue Responses to Prehydrated and Collagenated Cortico-Cancellous Porcine Bone Grafts: A Study in Rabbit Maxillary Defects

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ABSTRACT

Background: Bone substitutes should have osteoconductive properties and be completely replaced with new bone with time. Adding collagen gel to prehydrated and collagenated porcine bone (PCPB) particles results in a sticky and moldable material which facilitates clinical handling. However, the possible influence of the gel on the bone tissue response is not known.

Purpose: The objective of the study was to evaluate the bone tissue responses to PCPB graft with or without collagen gel and to evaluate the resorption/degradation properties of the biomaterials.

Materials and Methods: Fourteen rabbits were used in the study. Bilateral bone defects, $5 \times 8 \times 3$ mm, were created in the maxilla and filled with PCPB + collagen gel (test) or with PCPB only (control) and covered with a collagen membrane. Animals were killed after 2 ($n = 3$), 4 ($n = 3$), and 8 weeks ($n = 8$) for histological and morphometrical evaluations.

Results: There were no differences between test and control defects. Both materials showed bone formation directly on the particles by typical osteoblastic seams. The bone area increased with time (2–8 weeks) for both sides, from 16.2% (control) and 19.2% (test) to 42.7 and 43.8%, respectively. The PCPB, whether mixed with collagen gel or not, was resorbed by osteoclasts as well as part of remodeling with the formation of osteons within the particles. Morphometry showed a decrease of PCPB area from 19.4% (control) and 23.8% (test) after 2 weeks to 3.7 and 9.3% after 8 weeks, respectively.

Conclusions: Mixing collagen gel and PCPB to facilitate the clinical handling does not influence the bone tissue responses to the material, which exhibited osteoconductive properties and was resorbed with time.

KEY WORDS: animal model, bone healing, bone substitute, histology, xenograft

Bone substitutes are commonly used in oral surgery as alternatives to or together with autogenous bone grafts for minor reconstructive procedures. Such materials have been placed in bone defects/extraction sockets to facilitate healing, used as onlays in order to increase the width of the crest, or, more commonly, as inlays for augmentation of the maxillary sinus floor to enable implant placement.[1] Most bone substitutes are regarded as osteoconductive and serve as substrates/scaffolds for bone formation, that is, they support vessel ingrowth, cell migration/differentiation, and subsequent formation of new bone in osteogenic environments. With time, the interparticular spaces will be filled with newly formed bone and the bone substitute incorporated in the bone tissue. Some biomaterials will be completely resorbed with time, while others will remain more or less intact with time.

In general, bone substitutes have allo- or xenogeneic origins, or being synthetically made from calcium-based materials like calcium phosphate and calcium sulfate. Xenogeneic biomaterials are interesting as bone substitutes as they display a similar morphology as human bone and have the potential of being resorbed. Deproteinized bovine bone (DBB) is one of the most well-documented bone substitutes. This material has been

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shown to have osteoconductive properties and is well-incorporated in bone tissue as shown in both experimental and clinical studies.[2–4] Clinical histology has demonstrated integration of titanium implants in areas previously treated with bovine bone.[5,6] However, clinical studies also indicate that the material is not resorbable in a sense that it will completely disappear within a year.[7–9] Also, bone substitutes of porcine origin have been evaluated. Barone and colleagues[10] compared the use of autogenous bone alone or mixed with corticocancellous porcine bone (1 : 1) for maxillary sinus floor augmentation prior to implant placement in 18 patients. Biopsies were taken at implant placement 5 months later and processed for histology. There were no obvious differences between the two treatments, and the authors reported signs of resorption of the porcine bone particles. In a light microscopic and transmission electron microscopic (TEM) study of human biopsies from maxillary sinus augmented with porcine bone, Orsini and colleagues[11] demonstrated good biocompatibility of the material. TEM revealed a close contact between new bone and the porcine bone particles. However, there were no signs of ongoing resorption of the particles.

From a biological point of view, it seems that xenogeneic biomaterials work well, at least for augmentation of the maxillary sinus, although their resorption/degradation properties are still under debate. Most of these materials are available as granules and may be difficult to apply to the surgical site. Mixing with saline, blood, or fibrin glue may facilitate clinical handling. Moreover, the addition of collagen gel to the bone granules will create a sticky and moldable material, which simplifies application. However, the possible influence of the collagen gel on the bone tissue responses to the graft material is not known.

The purpose of the present experimental study was to evaluate the bone tissue response to prehydrated and collagenated porcine bone (PCPB), with and without collagen gel, when placed in defects in the rabbit maxilla, and to evaluate resorption/degradation properties of the biomaterial.

MATERIALS AND METHODS

Animals and Anesthesia

Fourteen adult (>7 months) female New Zealand white rabbits were used in the study. The animals were kept free in a purpose-designed room and were fed ad libitum with water and standard laboratory animal diet and carrots. Prior to surgery, the animals were given general anesthesia by an intramuscular injection of fluanison and fentanyl (Hypnorm®, Janssen Pharmaceutica, Brussels, Belgium) 0.2 mg/kg, and intraperitoneal injection of diazepam (Stesolid®, Dumex, Copenhagen, Denmark) 1.5 mg/kg body weight. Additional Hypnorm was added when needed. Local anesthesia was given using 1 mL of 2.0% lidocain/epinephrine solution (Astra AB, Södertälje, Sweden). Postoperatively, the animals were given antibiotics (Intenpencillin 2.250.000 IE/5 mL, 0.1 lm/kg body weight, LEO, Helsingborg, Sweden) and analgesics (Temgesic® 0.05 mg/kg, Reckitt and Colman, Wayne, NJ, USA) as single intramuscular injections for 3 days. The study was approved by the local committee for animal research.

Surgery

The bilateral edentulous areas between incisors and molars of the maxilla were used as experimental sites. The bone surface was exposed via a 10-mm-long incision between buccal and palatal mucosa. A mucoperiostal flap was raised. A 5×8 mm wide and 3 mm deep defect was drilled with the use of a 5 mm trephine drill followed by a large round burr (3 mm in diameter) under irrigation with saline (Figure 1A). The defects were filled with prehydrated and collagenated corticocancellous porcine bone (PCPB) particles (granulometry 250–1000 μm, GEN-OS, Tecnoss, Turin, Italy) or PCPB particles mixed with collagen gel (granulometry 600–1000 μm, MP3, Tecnoss) (Figure 1B). A collagen membrane (Evolution, Tecnoss) was placed over the defect (Figure 1C). The wounds were closed with resorbable sutures.

Three animals were killed after a healing period of 2 and 4 weeks, respectively. The remaining eight animals were killed after 8 weeks. The experimental areas were retrieved and immersed in 2.5% paraformaldehyde, 0.1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 24 hours. All specimens were X-rayed immediately after retrieval.

Tissue Processing and Analyses

Specimens were decalcified in ethylenediaminetetraacetic acid (15%) for a period of 2 weeks. Specimens were again X-rayed in order to verify the decalfication procedure. After dehydration in graded series of ethanol, the specimens were embedded in paraffine, sectioned

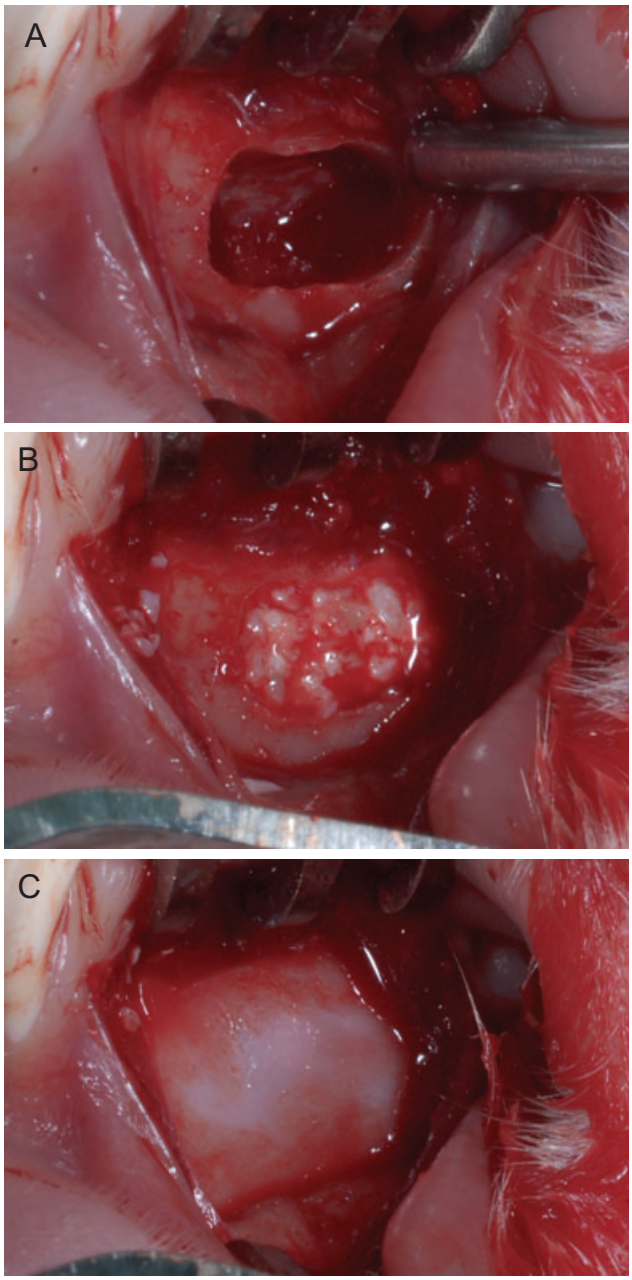

Figure 1 Clinical photos showing (A) defect in the rabbit maxilla, (B) defect filled with prehydrated and collagenated porcine bone, and (C) defect covered with a collagen membrane.

(3–5 μm sections), and stained with hematoxyline–eosine and modified Mallory aniline blue.

Examinations were performed in a Nikon Eclipse 80i microscope (Teknooptik AB, Huddinge, Sweden) equipped with an EasyImage 2000 system (Teknooptik AB) using ×1.0 to ×40 objectives for descriptive evaluation and morphometrical measurements. The histomorphometrical evaluations comprised measurements of the area of bone and porcine particles in relation to the total measurement area.

Statistics

The Wilcoxon signed-rank test was used to find possible differences between the two materials after 8 weeks of healing. A significant difference was considered if $p < .05$.

RESULTS

Clinical Findings

The postoperative healing was uneventful, and all animals fulfilled the predetermined healing periods. Clinically healthy mucosa without signs of infection covered the defects in all animals already after 5 to 6 days. Remnants of the resorbable suture could be seen after 2 weeks.

Histology

General Findings. A typical cross section comprised the medial/inferior bone wall, bordering the nose cavity and the palate, the central incisor superior in the section, and the experimental area (Figure 2). The collagen membrane was in general positioned along the lateral bone wall and the entrance of the defect. Ongoing resorption of the membrane was recorded in all specimens with infiltrating cells in the collagen membrane. The defect areas were occupied by PCPB particles, bone, and bone marrow in various degrees and in various degrees of maturation depending on time of healing. With time, vascularization proceeded showing mature vessels of all types (arterioles, venules, and capillaries) from week 4. In general, there were no obvious differences between the two materials.

Two Weeks. At this stage of the healing process, it was obvious that new bone formation already had started. Woven bone could be seen covering the collagenated porcine bone particles in both situations. Osteoblastic seams were also seen covering the woven bone, and microvessels started to appear in the soft tissue between the bone and the biomaterial (Figure 3, A and B).

Close to the membrane, a slight inflammatory response was recorded, but these cells did not penetrate into the defect. Obviously, this inflammatory response was a sign of degradation of the collagen membrane.

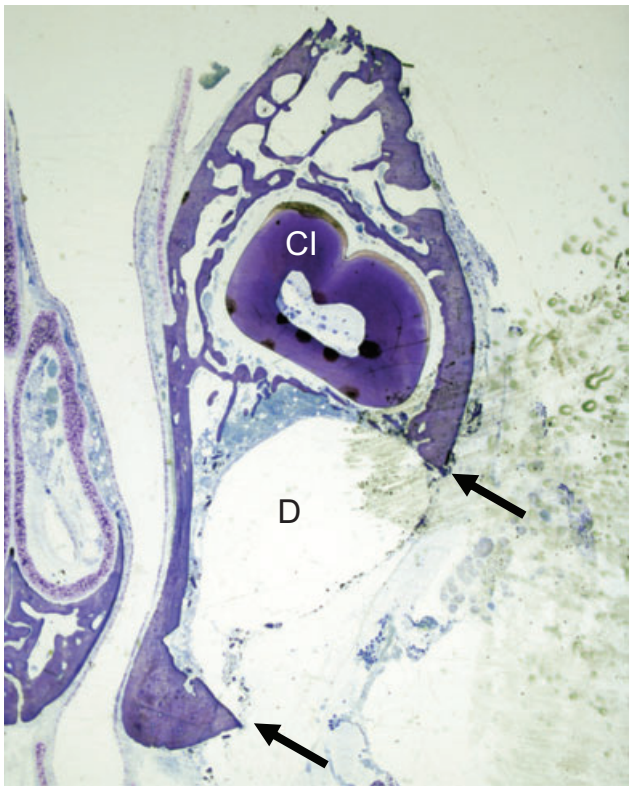

Figure 2 Cross section of the medial/inferior bone wall, bordering the nose cavity and the palate, the central incisor (CI) is seen superior in the section above the experimental area. Arrows show the entrance of the defect (D).

Four Weeks. At four weeks, the osteogenic activity was more pronounced (Figure 4A). Larger areas were covered with newly formed bone, and there was an active neovascularization going on in the defect. It was also found that an active resorption of the biomaterials was taking place (Figure 4B). There was no visual difference between the two materials tested.

Eight Weeks. At the end of the observation period (8 weeks), an active resorption was taking place in both materials tested (Figure 5A). The bone found in the defects was at 8 weeks more mature, and a reorganization of the bone was taking place (Figure 5B). All types of vessels were found both in the mineralized part and in the soft tissue. The collagen membrane was undergoing active degradation, and there were minor signs of inflammatory cells at the surface of the membrane (Figure 6).

Morphometrical Findings. The morphometrical measurements revealed an increased amount of mineralized bone with time with no significant difference between the two materials (Figure 7). In parallel, a similar decrease of the area of porcine bone was observed for both groups (Figure 8). No statistical difference was found regarding the resorption.

DISCUSSION

The present study was conducted to histologically evaluate the bone tissue responses to PCPB with or without collagen gel. The addition of collagen gel makes the graft material sticky, which facilitates its clinical use. According to the results, there was no obvious difference between the test and control materials. There were no signs of adverse reactions, and both osteogenesis and angiogenesis followed ordinary time frames.[12] Both graft

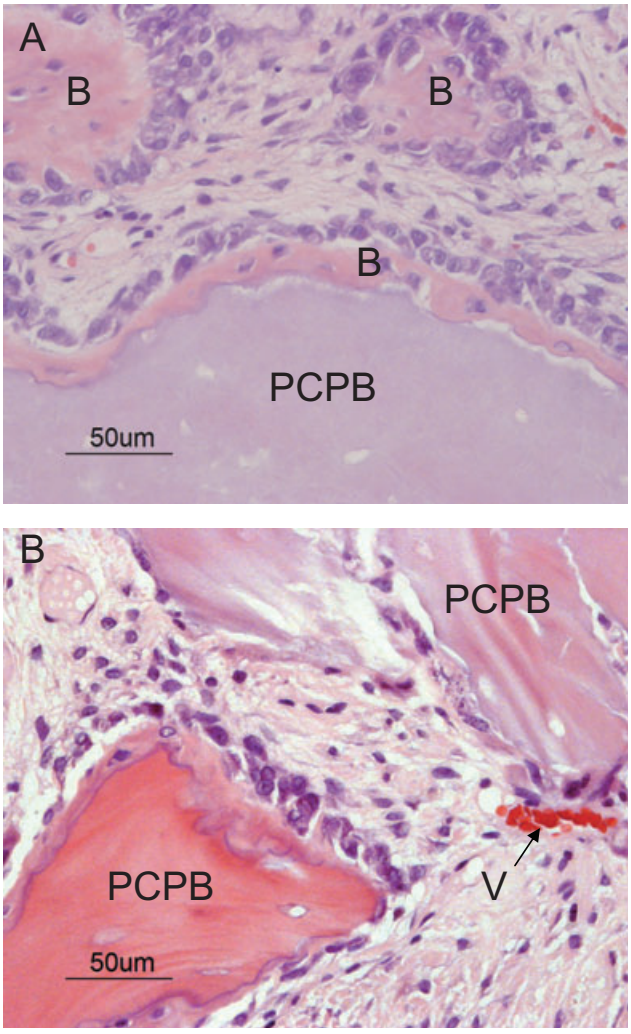

Figure 3 *A*, Micrograph showing prehydrated and collagenated porcine bone (PCPB) mixed with collagen gel (test) 2 weeks after insertion. Woven bone can be seen on the particles and ossification centers (B) in the marrow (V). *B*, Micrograph showing PCPB (control) 2 weeks after insertion. There is no difference when compared to (A) with respect to ongoing bone formation. To the right, a newly formed vessel can be seen (V arrow). Hematoxyline–eosine.

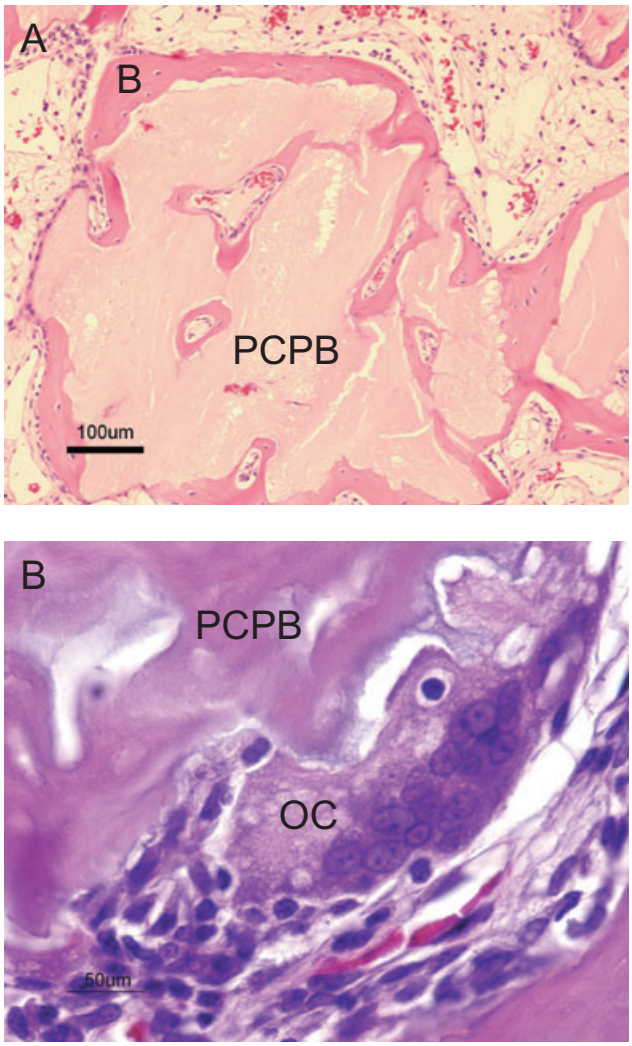

Figure 4 *A*, At 4 weeks, the osteogenic activity was more pronounced with no differences between test and control sites. Larger areas were covered with newly formed bone (B) and there was an active neovascularization going on in the defect. Particles of prehydrated and collagenated porcine bone (PCPB) totally surrounded by newly formed bone (B). Hematoxyline-eosine. *B*, Active resorption of the biomaterials by osteoclasts (OC) was taking place. Hematoxyline-eosine.

materials exhibited osteoconductive properties as bone formation with typical osteoblastic seams was observed directly on the surface of the grafted particles. The morphometric measurements showed increased bone area with time in parallel with a decrease of the graft area. This was most likely because of osteoclastic resorption because the presence of multinucleated cells in resorption lacunae at the surface of the PCPB particles was a common finding after 4 and 8 weeks. In addition, bone metabolizing units were seen within the granules, indicating remodeling and formation of osteons.

The present model has previously been used to study regeneration of defects with and without the use of barrier membranes, and to study the influence of mechanical trauma on bone density.[13–17] The defects will heal spontaneously within 4 weeks, but with a remaining buccal concavity.[13] In the present investigation, the model was used to study the bone tissue response to the PCPB material, but not to evaluate the effects on the overall morphology of the maxillary bone. A collagen membrane was used to cover the defect and to prevent migration of the particles. The histology showed that the membrane had fulfilled its function and was well-integrated with the overlaying soft tissues. The presence

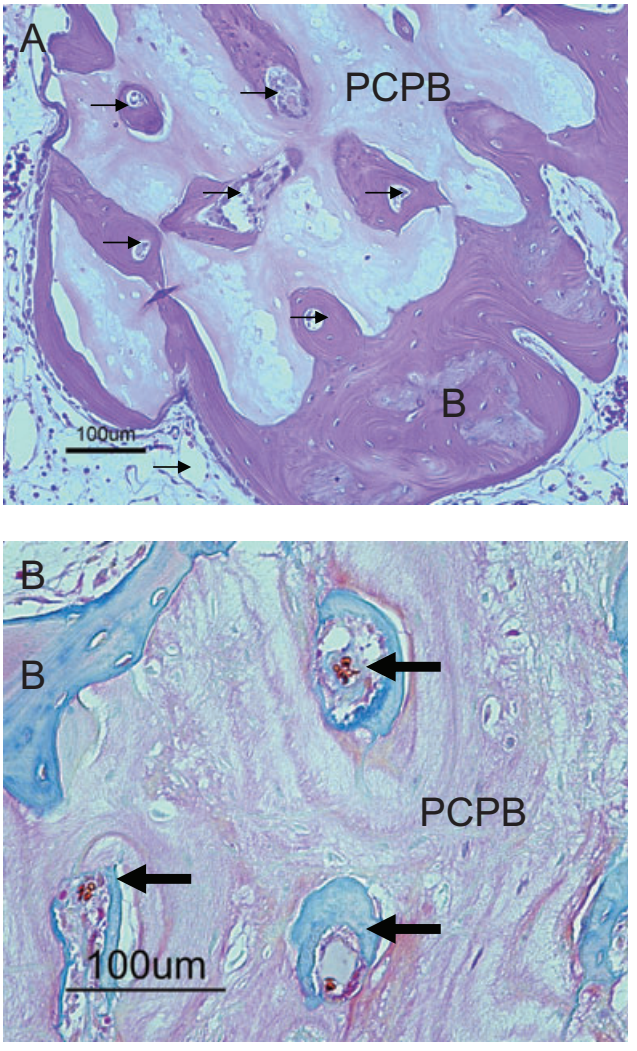

Figure 5 *A*, At the end of the observation period (8 weeks), an active resorption was taking place in both materials tested. Prehydrated and collagenated porcine bone (PCPB) mixed with collagen gel (test) showing the maturity of the newly formed bone (B). Note the high amount of neovessels within the bone and soft tissues (arrows). *B*, The bone found in the defects was at 8 weeks more mature, and a remodeling of the bone and PCPB was taking place. Arrows point at areas of newly forming osteons. B = bone modified Mallory alcian blue staining.

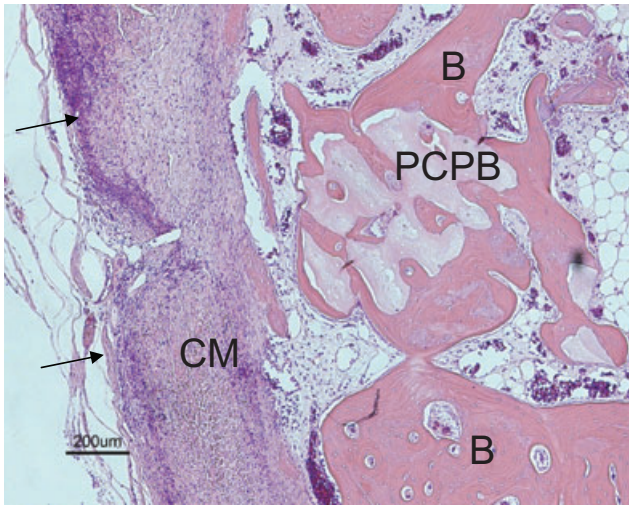

Figure 6 Micrograph showing the relationship between the collagen membrane (CM) and underlying tissues. A slight inflammatory reaction is seen (arrows), most likely being an active resorption of the membrane. A prehydrated and collagenated porcine bone (PCPB) particle is well-incorporated with newly formed bone (B).

of inflammatory cells of various kinds was evidently close to and in the membrane, and most likely took part in the degradation process.

Bone substitutes of xenogeneic origin are frequently used as grafting materials for filling bone defects and maxillary sinus floor augmentation procedures. DBB is probably the most common graft material, and it has been extensively investigated in both experimental and clinical studies. These studies have in general demonstrated good biocompatibility and osteoconductive properties of DBB as also demonstrated with histology of clinical biopsies.[6,8,18] However, it is still debated if the DBB will be resorbed with time or not. Animal investigations have demonstrated decreasing volumes and

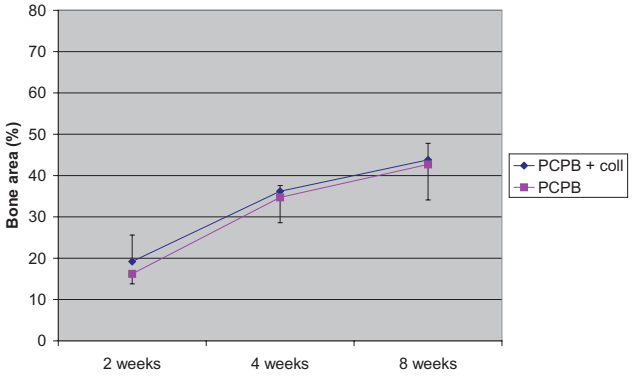

Figure 7 Graph showing the relationship between time, bone area in the defects, and the two materials.

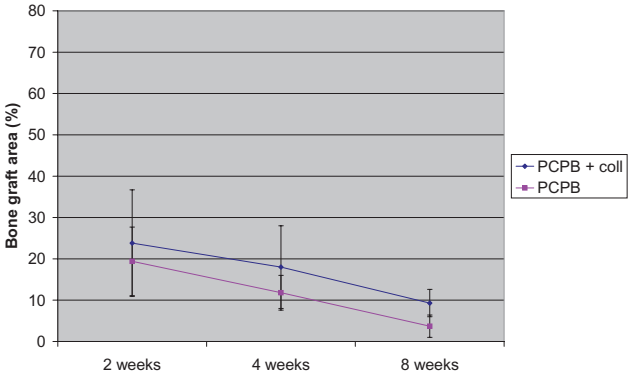

Figure 8 Graph showing the relationship between time, resorption of the biomaterials, and the two materials.

osteoclastic resorption,[4,19,20] while investigations using human biopsies with up to 6 years follow-up have shown large quantities of remaining DBB with no/few signs of resorption.[6,8,18] The present study clearly demonstrated resorption of the porcine bone particles. It is possible that the presence of collagen-induced adhesion of osteoclasts to the surface of the material. The mechanisms of osteoclastic resorption are not fully known. The cells have integrins which can link to certain proteins, for example, osteopontin, which may be important for adhesion and subsequent resorption.[21] One clinical study reported resorption of porcine bone based on histology of biopsies taken after 5 months sinus floor augmentation in 18 patients, which confirms our results.[10] However, this is in contrast with the findings of Orsini and colleagues[11] who could not observe resorption in clinical biopsies. Controlled clinical investigations with histology are obviously needed to establish the resorption properties of the porcine bone graft.

It is concluded that collagenated porcine bone exhibits good biocompatibility and osteoconductive properties, whether mixed with collagen gel or not. In this model, the material was resorbed by surface osteoclasts as well as part of remodeling with the formation of osteons.

ACKNOWLEDGMENTS

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